

EXPLORING THE ROLE OF NEURAL MIRRORING IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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ABSTRACT

Investigating the underlying neural mechanisms of autism spectrum disorder (ASD) has recently been influenced by the discovery of mirror neurons. These neurons, active during both observation and execution of actions, are thought to play a crucial role in imitation and other social-communicative skills which are often impaired in ASD. In the current EEG study, we investigated mu suppression, indicating neural mirroring in children with ASD between the age of 24 and 48 months and age-matched typically developing children, during observation of goal-directed actions and non-goal-directed mimicked hand movements, as well as during action execution. Results revealed no significant group differences with significant central mu suppression in the ASD children and control children during both execution and observation of goal-directed actions and during observation of hand movements. Furthermore, no significant correlations between mu suppression on the one hand and quality of imitation, age, and Social Communication Questionnaire (SCQ) scores on the other hand were found. These findings challenge the 'broken mirror' hypothesis of ASD, suggesting that impaired neural mirroring is not a distinctive feature of ASD.

Keywords: mirror neurons, ASD, mu suppression, EEG, children

INTRODUCTION

Autism Spectrum Disorder (ASD) represents a broad variation in symptomatology, ranging from rather mild to very severe symptoms in three separate domains: (a) impairments in social interaction, (b) communication, and (c) restricted and repetitive patterns of interest or behaviours (Wing, 1997). ASD has been characterized by various social-communicative dysfunctions (Williams et al., 2004). One frequently reported characteristic of ASD, also included in the diagnostic criteria of the DSM-IV-TR (American Psychiatric Association [APA], 2000), is an imitation impairment. This impairment, first reported by DeMyer and colleagues (DeMyer et al., 1972), is well documented (for a review, see Williams et al., 2004). However, the large variability in imitation skills in ASD, the variability across imitation tasks in research and the inconsistency of the definition of imitation all impede the development of a clear view on imitation in ASD (Vanvuchelen et al., 2011). Consequently, research on imitation in ASD is still debated and needs further exploration.

Given the central role of imitation in typical social-cognitive development (Meltzoff & Decety, 2003), it has been suggested that social-communicative symptoms in ASD could be the result of an imitation impairment reflecting a neurological deficiency (Rogers & Pennington, 1991). One commonly used explanation for the imitation impairment in ASD is the inability to map the perception of others into the observer's own system (Williams et al., 2004). This self-other mapping requires a match between observation and execution by which the motor knowledge of the observer is used to understand the observed action. This process is driven by 'an action observation/action execution matching system' (Gallese et al., 1996). A candidate neurobiological underpinning of this matching process is the mirror neuron system.

Mirror neurons were initially detected in area F5 of the macaque premotor cortex (Rizzolatti & Craighero, 2004). These neurons, distinguishable from other motor neurons, discharge when the monkey executes an action as well as when it observes another individual (human or monkey) performing a similar action (Di Pellegrino et al., 1992). The core idea is that the observation of an action leads to the activation of parts of the cortical neural network that is also active during action execution. Due to this neural mirroring, it is possible to accomplish automatic execution as well as simulation

of the observed actions. Impaired neural mirroring could lead to impaired self-other representations (Williams et al., 2001) and has been proposed to mediate the social and communicative deficits that characterize ASD (Oberman et al., 2008).

Contradictory to direct, single-cell neuron studies in monkeys indirect measures of the brain activity in humans using several non-invasive neurophysiological and brain imaging studies (e.g., Buccino et al., 2001; Fadiga et al., 1995; Hari et al., 1998) and behavioural measures such as gaze tracking (e.g., Falck-Ytter et al., 2006) have suggested the occurrence of a similar observation/execution matching system in humans. The first single cell study of Mukamel and colleagues (2010), using direct cellular activity, suggests the presence of multiple systems in the human motor cortex characterized by neural mirroring mechanisms. One commonly used non-invasive method for investigating human neural mirroring is analysing electroencephalographic (EEG) mu rhythm band oscillations (Muthukumaraswamy et al., 2004; Raymaekers et al., 2009). More specifically, resting motor neurons show spontaneous synchronization leading to a large amplitude of the EEG mu wave typically recorded in the 8-13 Hz frequency range in adults. Attenuation of the mu rhythm during motor activity is thought to reflect an increased activity level of these neurons and is also called 'mu wave suppression' (Gastaut et al., 1954). Similar mu wave suppression has been observed during the observation of actions performed by others as well as during motor activation (Gastaut & Bert, 1954). Without overtly reproducing the action, when humans observe someone performing an action, mirror neurons and motor areas are activated as if the observer is executing the observed action himself. This matching is thought to be an implicit, automatic, and unconscious process by which the internal motor knowledge of the observer is automatically activated and attributed during action observation (Fogassi, 2011; Gallese, 2003). Therefore, suppression of the mu wave rhythm typically recorded over the sensorimotor cortex has been argued to indicate a selective reflection of activity in neural mirroring areas (Pineda et al., 2000). A mu rhythm at a lower frequency range (between 6 and 9 Hz) with similar properties as the adult mu rhythm has been suggested in infants (Marshall & Meltzoff, 2011; Stroganova et al., 1999).

The discovery of mirror neurons and the pivotal role of imitation both in typical and atypical development have led to the hypothesis of dysfunctional mirror neurons in ASD (Williams et al., 2001). This dysfunction is likely to result in imitation and social-

communicative deficits often present in ASD (Fan et al., 2010; Williams et al., 2001). This hypothesis has been tested frequently but so far, evidence for the so called 'broken mirror theory of autism' seems inconsistent (Southgate & Hamilton, 2008). Several research findings support the idea of impaired mirror neuron functioning in ASD in adults (e.g., Bernier et al., 2007) and children. For example in the study of Oberman and colleagues (2005), individuals with ASD between 6 and 47 years old showed significant mu suppression during self-performed hand movements, but not during movement observation. These findings support the idea of broken mirror neurons in ASD which was also the case in the study of Martineau and colleagues (2008), where 5-year-old autistic children showed no mu suppression during action observation. Additionally, Dapretto and colleagues (2006) found in their fMRI study support for dysfunctional neural mirroring mechanisms during both imitation and observation of emotional expressions in ASD children. Impaired mirror neuron functioning in this study was negatively correlated with symptom severity in children with ASD which may influence social deficits often observed in ASD. On the other hand, Oberman and colleagues (2008) measured significant mu suppression during action observation in individuals with ASD under specific conditions such as the use of a familiar hand model. In addition, Raymaekers and colleagues (2009) found equally strong mu suppression during both self-performed and observed hand movements in children between 8 and 13 years with high functioning autism compared to the control group. Similarly, also Fan and colleagues (2010) found in their study that individuals with ASD showed mu suppression similar to the control group during the observation of hand actions. Hence, to date, there is insufficient support for the broken mirror theory of autism (see Gallese et al., 2011 for an overview of this discussion; Southgate & Hamilton, 2008).

Given the pivotal role of imitation in early social-cognitive development, especially in infancy and early childhood (Rogers & Pennington, 1991), and given the effect of experience on neural mirroring activity (e.g., van Elk et al., 2008), it is indicated to investigate neural mirroring in younger children with ASD than has been the case thus far. If a dysfunctional neural mirroring system is causing or mediating the cascade of social-communicative and social-cognitive deficits in ASD, it should be clearly evident in infants and young children with ASD. Also, at this age, the limited social experience

some children with ASD have, should play a smaller role than it does in adolescence or adulthood.

Therefore, the current study aimed to explore neural mirroring in young children with ASD and in typically developing controls, all between 24 and 48 months old. We used mu suppression as indicator of activity in the mirror neurons during the observation of goal-directed actions and non-goal-directed mimicked hand movements and during action imitation. The present study examined following research questions: (1) Do young children with ASD (age 24-48 months old) show central mu suppression during the observation of goal-directed actions compared to a matched control group of typically developing children? According to the broken mirror hypothesis in ASD (e.g., Martineau et al. 2008; Oberman et al., 2005), we may expect a lack of or diminished mu suppression during the action observation condition in the ASD group. (2) Do children with ASD and typically developing children (age 24-48 months old) show central mu suppression during the observation of intransitive or mimicked (non-goal-directed) actions? In adults, motor cortical modulation has been observed during the observation of transitive (goal-directed) as well as intransitive or mimicked hand actions (e.g., Fadiga et al., 2005; Maeda et al., 2002). To date, only a few studies investigated neural mirroring activity during hand movement observation in typically developing children (e.g., Southgate et al., 2010; Warreyn et al., 2013) but not in children diagnosed with ASD. The study of Warreyn and colleagues (2013) suggested that, similar to adults, 18- to 30-month-olds do show neural mirroring activity during the observation of intransitive or mimicked hand movements, while this is not yet the case in younger infants (e.g., Southgate et al., 2010). Therefore, one of the aims of this study was to investigate the role of goal-directedness of actions on mu suppression in older children with and without ASD by including intransitive hand movement actions in our paradigm. In line with the idea of impaired mirror neuron functioning in ASD, we may expect less mu suppression during this observation condition in the ASD group. Additionally, we investigated if children with ASD (age 24-48 months old) show central mu suppression during the execution of goal-directed actions compared to a matched control group of typically developing children? We may expect (equally strong) suppression of mu oscillations during action execution in both groups as there is no evidence for diminished activity in sensorimotor areas during movement execution in ASD (Bernier et al., 2007).

(3) Is mu suppression related to child characteristics such as quality of imitation, chronological age and/or symptomology measured by Social Communication Questionnaire (SCQ) scores?

MATERIAL AND METHODS

Participants

The initial sample consisted of 35 children with ASD and 42 typically developing (TD) control children. From this sample, 17 participants with ASD and 23 typically developing children were excluded prior to analyses due to limited cooperation (ASD: $n = 4$; TD: $n = 2$), insufficient artefact free data (ASD: $n = 13$; TD: $n = 19$) or technical problems with the EEG equipment (TD: $n = 2$). As a result, the final sample for further analyses was composed of 18 ASD children and 19 typically developing children (mean age = 41.94, $SD = 13.80$). The groups differed on gender, ($\chi^2(1) = 4.88, p = .027$) but did not differ on chronological age, $F(1,35) = 0.06, p = .808$. The ASD group scored significantly higher on the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) than the TD group ($t(26) = -5.16, p < .001$). Table 1 presents the characteristics of the participants. Information about handedness was gathered through parent report.

ASD subjects were recruited through Belgian Government certified University Clinics for Developmental Disorders and multiple treatment centres for developmental disorders. All ASD participants were examined and formally diagnosed with 'Autism Spectrum Disorder and/or 'Pervasive Developmental Disorder - Not Otherwise Specified' according to the DSM-IV-TR (American Psychiatric Association [APA], 2000), independently by a qualified multidisciplinary team of specialists who were all familiar with ASD.. One of the tests included in the diagnostic protocol was the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 1999). Except for two participants, the diagnosis was confirmed with the ADOS as the ASD children scored above the cut-off for ASD. Control subjects were recruited through Flemish day-care centres and several

magazine or website advertisements. For each participant, parental informed, signed consent was required.

INSERT TABLE 1 ABOUT HERE

General procedure

The children were tested in a quiet laboratory room at the university. The experiment started with a free play moment with some attractive toys in order to let the child get used to the environment and experimenters. Experimenter 1 (also the demonstrator of the actions during the test phase) played with the child, while experimenter 2 prepared the appropriate EEG cap. Meanwhile, the procedure was explained to the parent. After the placement of all the electrodes in the appropriate EEG cap, the parent was asked to sit at the table together with his/her child. To maximize attention and to minimize movement, the child was seated on its parent's lap throughout the entire test phase. Subsequently, the experimenters placed the EEG cap on the child's head while the child was watching a popular cartoon movie. Once the EEG cap was in place, electrolytic conducting gel was applied with a syringe in each active electrode in order to obtain a good EEG signal. White curtains surrounded the laboratory room to minimize visually distracting environmental influences. A white roller blind, attached on a wooden frame, went up and down between the different conditions. The objects and actions were demonstrated at a viewing distance of approximately 60 cm. Parents were asked to be as quiet as possible during the EEG recording period in order not to distract the child or influence the measurement. The experiment was videotaped by 2 cameras (one focusing on experimenter 1 and one focusing on the participant) in order to code the child's behaviour afterwards.

EEG imitation and observation paradigm

EEG data were collected during 4 experimental conditions with 5 different objects: (1) *Object observation condition*: Each testing phase started with the presentation of a dangling object, moving back and forth in a non-goal-directed way. During this condition, the experimenter was hidden behind the white curtain. This

observation condition was used as a baseline condition based on the assumption that the subjects had no prior experience with the objects. Each following experimental condition was compared with this baseline condition. (2) *Action observation condition*: The experimenter demonstrated a simple goal-directed action (with an observable end-state) with each object and a white box (for example, a small toy hippopotamus, starting from one side of the box, was brought in a wave-movement into the box; a toy loupe was picked up and brought in a wave-movement to the other side of the box.). The experimenter said: 'look <name child>' and made eye-contact with the child to ensure that the subject was attentive to the demonstration. In order to obtain a sufficiently long interval EEG data and to avoid effects of one-sided lateral presentation, each action was demonstrated three times with the left hand and three times with the right hand. The starting hand was counterbalanced between the different objects. (3) *Action imitation condition*: After modelling the action, the child was given the object and the box, and asked to imitate the observed action. Participants were encouraged (non)-verbally in a non-specific way to imitate and were given as much time as needed to perform the actions themselves. (4) *Hand movement condition*: Mimicked actions were demonstrated during this fourth condition. The experimenter executed hand movements identical to those during the action observation condition but now without the objects and without direct reference of gaze towards the child which makes this condition less social. Subjects were expected only to observe those actions, not to imitate them. Again, the hand movements were demonstrated 3 times with the left hand en 3 times with the right hand.

Each experimental session started with the object observation condition (baseline condition) for all 5 objects subsequently. The order of the other three experimental conditions was counterbalanced across subjects, with the limitation that the action imitation condition always followed the action observation condition so that the participants first observed what they had to imitate. The order in which the objects were presented was fixed for each participant. Each demonstrated action lasted about 30 seconds per object, which resulted in a total duration of about 20 minutes for the entire session. After the EEG recording and the test phase, the parents were debriefed and they received a gift card as reward for their participation. Finally, the parents were asked to fill in the Dutch version of the MacArthur-Bates Communicative Development

Inventories (N-CDI, Zink & Lejaegere, 2002; original version Fenson et al., 1993) and the Social Communication Questionnaire (SCQ; Rutter et al., 2003; Dutch translation by Warreyn et al., 2004) at home.

EEG data recording

EEG was recorded from 32 active Ag/AgCl electrodes placed according to the international 10-20 system (Jasper, 1958), relative to an average reference. The electrodes were embedded in a child-friendly stretch EEG-cap with a ground electrode placed at AFz (EasyCap, Brain Products, GmbH, Munich, Germany). EEG was recorded by the use of an EEG-amplifier (QuickAmp) with a sample rate of 500 Hz, 1 s time constant, a low pass filter of 70 Hz, and a notch filter of 50 Hz. Horizontal electro-oculogram (HEOG) electrodes were placed at the left and right outer canthi of the eyes. Vertical EOG was calculated by comparing the recording of an electrode above the eye, at position Fp2, with the common reference. Initially VEOG was computed by comparing Fp2 with an electrode placed below the left eye, but many children did not tolerate this additional electrode. After comparing corrected data with this electrode and the common reference method, no significant differences occurred between these two calculations. Inter-electrode impedance was measured and confirmed to be below 10 k Ω for all electrodes. To synchronize the EEG recordings and video recordings, a button was pushed at the beginning of each experimental condition. This button sent a marker to the recorded EEG signal and simultaneously emitted a LED light which was visible on both video-cameras.

Offline behaviour coding

After the experiment, the video recordings were coded offline with The Observer XT 9.0. (Noldus Information Technology, 2009). First, the four experimental conditions were marked by start and stop codes. Second, we coded attentive behaviour (whether or not the child was watching the presentation) in the observation conditions, imitative behaviour in the action imitation condition, and vocalization and motor movements within each experimental condition. Further analyses were only based on the sections where the child was quietly attending the demonstrations (during the object

observation, action observation, and hand movement condition) and was actually imitating during the action imitation condition. During this behaviour coding, fragments with too much motor and/or vocalization codes were excluded in order to minimize contamination of the EEG data. Obviously, it was impossible to exclude all these segments. Therefore, an additional exclusion of motor movements and vocalizations was performed afterwards through the artefact rejection procedure during the EEG analyses.

Furthermore, quality of imitative behaviour was coded by an observer who was blind for group membership. Three criteria were assigned to each performed action. The child received one point for every criterion he/she met (with a maximum of three points per imitation attempt). For every object, the best attempt was identified. A mean imitation quality score was calculated, averaging the best scores for the 5 objects. In the ASD group, the mean imitation score was 2.34 ($SD = .45$). This was comparable to the imitation score of the control group ($M = 2.44$, $SD = .39$; $t(35) = .72$, $p = .476$). To assess inter-observer reliability, an independent coder double-coded 25% randomly selected videos. This resulted in a Cronbach's Alpha Coefficient of .94 (Cronbach, 1951).

EEG data processing

Brain Vision Analyzer (Brain Products, 2007) was used for offline analyses of the recorded raw EEG data. Based on the assumption that mu rhythm is measured over the sensorimotor cortex (Marshall et al., 2011; Muthukumaraswamy et al., 2004), EEG power recorded from electrode positions C3 and C4 was further investigated. First, the raw EEG data were visually inspected to exclude contaminated signals due to artefact influences. Afterwards, EEG was re-referenced to the average reference with exclusion of the most disturbed electrode channels. EEG data were filtered with a high pass filter of 0.1 Hz, a low pass of 30 Hz, and a 50 Hz notch-filter. Furthermore, the Gratton and Coles algorithm correction was applied to correct for horizontal and vertical eye movements (Gratton et al., 1983). The remaining data were segmented in 1s-epochs with 50 % overlap. Bad segments were removed with an artefact rejection algorithm using a maximal allowed voltage step of 100 μV per sampling point, a maximal allowed absolute difference of 400 μV between two values in the segment, and an activity of 0 μV during maximum 100 milliseconds. This resulted in an average of 226.45 segments

($SD = 100.66$) per child per condition. There was no significant difference between both groups in the number of artifact free segments during the three conditions with $t(35) = 1.08$, $p = .287$ for the hand movement condition, $t(35) = -.09$, $p = .931$ for the action observation condition and $t(35) = -.97$, $p = .339$ for the action imitation condition. On the remaining segments, a Fast Fourier Transform was performed with a Hanning window of 10 %, averaged for each experimental condition. In analogy with previous studies, the individual mu frequency was identified by subtracting the baseline condition from the action imitation condition for each subject individually (e.g., Lepage & Théoret, 2006; Muthukumaraswamy et al., 2004). The individual mu frequency band was defined as the 3-Hz interval around the highest peak value of that subtraction at the central electrode positions. The mean mu frequency of the total sample was 8.58 Hz ($SD = .67$), which is in agreement with previous studies on mu/alpha rhythm frequencies in infants (Marshall et al., 2002; Stroganova et al., 1999).

The procedure of Oberman and colleagues (2005) was used to calculate the mu suppression values. To control for variability due to possible individual differences (e.g., scalp thickness or electrode impedance), we used a ratio to estimate the relative power for each condition. More specifically, we calculated the ratio of the power during the condition of interest (action observation, hand movement, or action imitation) relative to the power during the baseline condition (object observation). Since the ratio data were non-normally distributed, the log transform of each ratio was estimated. This resulted in a value representing mu suppression (i.e., a negative value), mu enhancement (i.e., a positive value) or no suppression (i.e., a value of zero).

RESULTS

Independent sample t-tests were performed to evaluate whether the order of presentation affected mu suppression. This was not the case (all $.68 < t(16) < .79$, all $p > .05$ in the ASD group and $.27 < t(17) < .96$, all $p > .05$ in the TD group). Therefore, the order of presentation of the conditions was not further included in the analyses.

Mu suppression

An overall 3x2x2 repeated-measures ANOVA was conducted with condition (hand movement, action observation, and action imitation) and hemisphere (C3 and C4) as within-subjects factors and group (TD and ASD) as between-subjects factor. Results revealed no significant main effects of group $F(1,35) = 1.38, p = .248$, condition $F(2,34) = 1.59, p = .218$ or hemisphere $F(1,35) = .99, p = .326$ and no significant interaction effects, with $F(2,34) = 1.59, p = .219$ for condition by group, $F(2,34) = 1.68, p = .202$ for hemisphere by condition and $F(1,35) = 1.36, p = .252$ for hemisphere by group. No significant 3-way interaction effect was found between condition, hemisphere and group, $F(2,34) = .61, p = .550$.

Furthermore, one sample t-tests revealed significant central mu suppression (i.e., mu suppression assembled over electrode positions C3 and C4) during the hand movement condition, action observation, and action imitation condition in both the ASD group and the TD group, with $t(17) = -3.99, p = .001$; $t(17) = -4.29, p < .001$; and $t(17) = -3.71, p = .002$ respectively for the ASD group and $t(18) = -4.02, p = .001$; $t(18) = -3.55, p = .002$; and $t(18) = -2.37, p = .029$ respectively for the TD group. The means and standard deviations of the mu suppression at electrode positions C3 and C4 separately and averaged as overall central mu wave activity are presented in Table 2.

INSERT TABLE 2 ABOUT HERE

Additional analyses of electrode activity recorded from an occipital electrode (Oz) were conducted to assure that the observed central suppression was related to the mu rhythm and not to posterior alpha activity. The total sample showed no suppression at Oz during action imitation in the frequency band under investigation, $M = .01, SD = .42$; $t(32) = 0.14, p = .889$. Furthermore, during action imitation, a paired sample t-test revealed significantly stronger central suppression ($M = -.26, SD = .39$) compared to occipital suppression ($t(32) = 2.84, p = .008$). This suggests that the observed mu

suppression was specific to the central electrode positions and was not the result of overlapping occipital activity.

Relationship between mu suppression and imitation, chronological age, and SCQ scores

Pearson's correlation coefficients were calculated to investigate the relations between central mu wave suppression on the one hand and quality of imitation, chronological age, and SCQ-scores on the other hand. Both the ASD group and the TD group showed no significant correlation between central mu suppression and quality of imitation with small correlations ranging from $-.03 < r < .21$, all $p > .05$ in the ASD group and $-.07 < r < .04$, all $p > .05$ in the TD group. In the ASD group, age only correlated significantly with central mu suppression during the hand movement condition with a medium correlation of $r = -.54$, $p = .020$. Furthermore, both groups demonstrated no significant correlations with chronological age, reflected in small correlations of all $-.23 < r < -.01$, $p > .05$ in the ASD group and all $-.01 < r < .09$, $p > .05$ in the TD group. Central mu suppression during action observation in the TD group correlated marginally significant with SCQ scores with a medium correlation of $r = -.44$, $p = .088$. Central mu suppression during the other conditions in the TD group and during all conditions in the ASD group did not correlate significantly with SCQ scores, with small correlations between $-.07 < r < .23$, $p > .05$. See Table 3 for details.

INSERT TABLE 3 ABOUT HERE

DISCUSSION

The aim of the current study was to investigate mu suppression, as a measure of neural mirroring during imitation and observation tasks in children diagnosed with ASD and typically developing controls, all between 24 and 48 months old. Concerning our first two research questions, results revealed significant central mu suppression in both groups during the observation of goal-directed actions and hand movements. The

occurrence of mu wave suppression during the observation of non-goal-directed hand movements in both groups, suggests that the observation of motor movements alone without objects is sufficient to induce neural mirroring activity in children with and without ASD (Maeda et al., 2002). Additionally, we found (equally strong) mu suppression during the action imitation condition in both groups. Furthermore, no differences were found between both groups regarding overall mu suppression. Together with the absence of a group difference in central mu suppression, these results are in line with the idea of an intact action observation/action execution matching system in children diagnosed with ASD and argue against the broken mirror hypothesis of ASD (Hamilton et al., 2007;; Oberman et al., 2008; Southgate & Hamilton, 2008). The finding that in infants and young children with ASD, mu suppression during the observation of goal-directed as well as mimicked actions is as strong as in typically developing infants and young children, suggests that it is unlikely that impaired mirror neuron functioning is causing the social-communicative and social-cognitive impairments in ASD.

Finally, Pearson's correlation coefficients were calculated and revealed that central mu suppression in both groups was not correlated with quality of imitation. This does not support a strong relationship between neural mirroring and imitation capacities, as has been hypothesized (Rizzolatti & Craighero, 2004). However, it is possible that our imitation measure was not sensitive enough, or that our sample was not diverse enough to detect possible correlations. This can also be an explanation for the lack of significant correlations with chronological age. Only the ASD group showed a significant correlation between age and central mu suppression during the hand movement condition. It is possible that the age range of 2 years within our participant group may be too small to detect significant correlations between age and mu suppression in the TD group and in the other conditions in the ASD group. Additional research is needed to replicate mu suppression during observation and imitation tasks, over different time periods in individuals with ASD. This could give more information about stability or evolution of neural mirroring in ASD. Except for a trend between central mu suppression during action observation and scores on the SCQ in the TD group, no significant correlations were present in both groups. Although the ASD group scored significantly higher on the SCQ compared to the TD group, mu suppression did not correlate significantly with SCQ-scores in this clinical group. The lack of substantial

correlations between mu suppression on the one hand and social-communicative abilities typically impaired in ASD on the other hand also provide no support for the broken mirror hypothesis of autism.

Some limitations of this study can be mentioned. A first critical remark concerns the sample of the current study. Our sample of ASD participants excluded children with a severe developmental delay, which makes this sample not completely representative for the general ASD population. However, this study wanted to investigate neural mirroring in children with ASD, independently from developmental delay. Additionally, the sample size was rather small. It is possible that the small and medium correlations are related to the sample size of the investigated groups (Kareev et al., 1997). Therefore, this study needs to be replicated with larger samples. Furthermore, both participant groups were not matched on gender. However, previous adult research suggested gender differences concerning neural mirroring with more mu suppression in girls compared to boys during action observation (e.g., Cheng et al., 2008; Cheng et al., 2006) resulting from stronger empathic feelings of women during the observation of other's actions. Although it is assumed that infants can demonstrate empathic behaviours (Rieffe et al., 2010) with empathic markers already present at 8- and 10-months of age (Roth-Hanania et al., 2011), gender differences in empathic behaviour are not consistently found in infancy. Furthermore, it is assumed that gender differences in empathy may become more pronounced and stable as infants grow older (Roth-Hanania et al., 2011). Therefore, future research needs to focus on the relationship between gender and mu suppression in different infant participant groups matched for gender. On the other hand, since there were less girls in the ASD group, it is highly unlikely that the significant mu suppression measured in this group is due to a larger proportion of boys. On the contrary, with a similar proportion of girls in the ASD group, if any change would be observed, it would be more likely to be stronger mu suppression. Second, simple imitation tasks were used with clear instructions by which the participants were explicitly asked to imitate. However, it would be interesting to investigate neural mirroring in ASD during spontaneous imitation, without clear or explicit instructions. Additionally, imitation requires more than only mapping of observed visual information to execute motor output (Southgate & Hamilton, 2008). Other cognitive processes such as motor control or visual analyses are needed to perform correct imitative behaviour (Tessari & Rumiati, 2004). Therefore, it would be interesting to include different types of

imitation tasks in future research about neural mirroring in ASD. In this way, it could be investigated if mirror neurons or other processes respond differently depending on the task variability (Williams, 2008). Third, we did not take into account whether the children diagnosed with ASD followed therapy or intervention programs outside the research project which could have influenced their imitation abilities. It is possible that this was the case, since we found no group difference in imitation quality. However, various studies found intact imitation abilities in ASD (e.g., an intact ability to imitate object-oriented and goal-directed actions; for a review, see Hamilton, 2008 or good performance of explicit imitation tasks; Beadle-Brown & Whiten, 2004). Finally, it was difficult to apply event-related time frequency analyses in the present study because of the use of an ecological valid paradigm, with live stimuli presented during rather long time intervals. During the analyses, fragments with too many motor and/or vocalization codes were excluded and only the segments where the child was quietly attending the demonstrations during the object observation, action observation, and hand movement condition were further used based on the method of previous research using the same stimuli and paradigm (e.g., Ruysschaert et al., 2013; Warreyn et al., 2013). However, event-related time frequency analyses make it possible to investigate temporal changes of the mu rhythm during a specific time period. Therefore, future research should try to develop paradigms using live stimuli combined with the possibility to use event-related time frequency analyses to investigate the temporal changes of the mu rhythm within a particular time period.

In summary, the present study investigated mu suppression, as an index of neural mirroring, in infants and young children with ASD, compared to typically developing controls. Significant mu suppression in the ASD group during both observation and imitation conditions, together with the absence of a group difference in mu suppression and only limited correlations between mu suppression and child characteristics argue against the hypothesis of deficient neural mirroring systems in ASD. Since this is the first study to investigate neural mirroring in ASD at this young age, future studies should try to replicate these findings in larger and perhaps more heterogeneous samples. In addition, the effect of social and motor experience on the development of neural mirroring should be investigated, preferably by means of longitudinal studies enabling us to disentangle possible causal effects.

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The procedure used in the current study was approved by the ethical committee of Ghent University. Informed consent was obtained from all subjects.

The authors declare that they have no conflict of interest.

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Table 1. *Subject characteristics*

	ASD group (<i>n</i> = 18)	TD group (<i>n</i> = 19)
Chronological age (months)		
Mean (<i>SD</i>)	42.52 (13.72)	41.39 (14.23)
Age Range	25.90-60.00	25.20-58.70
Language mean age (months)		
Receptive (<i>SD</i>)	39.85 (12.89)	45.63 (16.72)
Expressive (<i>SD</i>)	38.77 (14.47)	44.94 (18.62)
SCQ mean (<i>SD</i>)	13.42 (4.68)	5.00 (3.95)
Gender ratio M : F	14 : 4	8 : 11
Handedness (R : L : ambi)	15 : 1 : 2	12 : 5 : 2

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn et al., 2004).

Table 2. *Mu suppression for both groups at each electrode position separately and assembled during each condition*

		ASD group (<i>n</i> = 18)		TD group (<i>n</i> = 19)	
		<i>M</i> (<i>SD</i>)	<i>t</i> (17)	<i>M</i> (<i>SD</i>)	<i>t</i> (18)
C3	HM	-.24 (.22) ***	-4.50	-.23 (.31) **	-3.17
	AO	-.24 (.19) ***	-5.19	-.16 (.19) ***	-3.78
	AI	-.24 (.37) *	-2.76	-.28 (.55) *	-2.19
C4	HM	-.20 (.33) *	-2.61	-.12 (.25) *	-2.08
	AO	-.23 (.32) **	-3.01	.01 (.16)	0.15
	AI	-.31 (.38) **	-3.49	-.23 (.45) *	-2.21
C	HM	-.22 (.23) ***	-3.99	-.17 (.19) ***	-4.02
	AO	-.23 (.23) ***	-4.29	-.08 (.10) **	-3.55
	AI	-.28 (.32) **	-3.71	-.25 (.46) *	-2.37

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; C3 = mu suppression at electrode position C3; C4 = mu suppression at electrode position C4; C = mean central mu suppression assembled over electrode position C3 and C4; HM = mu suppression during the hand movement condition; AO = mu suppression during the action observation condition; AI = mu suppression during the action imitation condition.

p* ≤ .05; *p* < .01; ****p* ≤ .001.

Table 3. *Overview of Pearson correlations between central mu suppression and imitation, chronological age and SCQ scores*

	ASD group (<i>n</i> = 18)			TD group (<i>n</i> = 19)		
	Quality imitation (<i>r</i>)	CA (<i>r</i>)	SCQ (<i>r</i>)	Quality imitation (<i>r</i>)	CA (<i>r</i>)	SCQ (<i>r</i>)
CHM	-.020	-.541*	-.061	-.068	-.008	.090
CAO	.201	-.220	-.032	-.030	.078	-.440°
CAI	-.016	-.007	.111	.037	.085	.226

Note. ASD group = children diagnosed with ASD; TD group = typically developing group; CHM = mu suppression at central electrode positions during the hand movement condition; CAO = mu suppression at central electrode positions during the action observation condition; CAI = mu suppression at central electrode positions during the action imitation condition; CA = chronological age; SCQ = total score on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; Dutch translation by Warreyn et al., 2004).

°*p* < .10; **p* < .05.